

POLLINATION AND REPRODUCTION IN NATURAL AND MITIGATION POPULATIONS OF THE MANY-STEMMED DUDLEYA, *DUDLEYA MULTICAULIS* (CRASSULACEAE)

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ABSTRACT

We investigated the reproductive biology of the rare and endangered plant, *Dudleya multicaulis* at five separate sites, three natural and two mitigation sites. We employed dawn to dusk observations to determine the spectrum of pollinators visiting *D. multicaulis*, took pollen samples from visitors to determine floral constancy, sampled nectar to determine volume produced per flower, examined the number of flowers per inflorescence, the number of those flowers that produced seed, and total seed set to determine reproductive output, completed seed germination tests to determine viability, and transplanted germinated seedlings from Petri dishes to soil to determine how well seedlings survive transplanting. *Dudleya multicaulis* was visited by flower beetles, native and European honey bees, flies, and a variety of other insects. Nectar production per flower averaged 0.12  $\mu$ l. Bees averaged 99% floral constancy to *D. multicaulis*. Reproductive output measured by flower production and fruit/seed set were not significantly different among sites. Among all populations, the average fruit set ranged from 86.9 to 94.4%. The large fruit set coupled with the diversity of floral visitors suggests that *D. multicaulis* is not pollinator limited. Data suggest that *D. multicaulis* is capable of self-pollination in absence of vectors. Seed germination and transplanted seedling survival did not differ significantly among sites. Results suggest that sowing seed may be better for plant establishment rather than transplanting when mitigation is necessitated.

Key Words: Auto-fertility, *Dudleya multicaulis*, pollination, reproductive output, seedling survival, transplanted.

Information on the reproductive biology of rare plants can provide some assistance in understanding why some plants are rare and others are common (Kearns et al. 1998). Of special importance are cases where rare plants, which are to be extirpated as a result of development, are physically transplanted to new sites or seeds from existing populations are sown in new locations intended to serve as mitigation sites. Data relative to the reproductive biology of such species should play a significant role in decision-making regarding the management, salvaging, and moving of such rare plants as part of a mitigation process. Information of this type may indeed prove critical to the success or failure of the establishment of salvaged plants or seeds in mitigation areas.

*Dudleya multicaulis* (Rose) Moran (Crassulaceae), the many-stemmed Dudleya, is recognized as a rare and endangered plant in California and elsewhere (List 1B.2) by the California Native Plant Society (CNPS 2005). As part of the mitigation process necessitated by the Final Project Environmental Impact Report for the Santiago Hills II Planned Community and certified by the City of Orange in 2000 (Homrighausen unpublished), this sensitive species was transplanted or seeded to new areas as part of a pilot study for future mitigation. Mitigation sites

were selected based on “their similarity to the existing population sites in terms of vegetation composition and cover, apparent soil type, and depth, slope, and aspect” (Homrighausen unpublished).

A patchily-distributed geophyte, *D. multicaulis* is typically associated with the coastal sage scrub plant community of southern California (Dodero 1995; Marchant et al. 1998). Little is known about its reproductive biology (RCIP 2003), although several possible bee, fly and flower beetle pollinators are projected to be involved (Dodero 1995).

To provide information relative to the reproductive biology of this rare species, we observed the developmental sequence of flowering and investigated the pollination biology of this species during the peak flowering period in May of 2005 at the Santiago Hills site (Jones, Shropshire, and Allen unpublished), which is within the Santiago Hills II Planned Community and the East Orange development projects (Homrighausen unpublished). Subsequently, we examined the reproductive output, seed germination, and seedling survival and reproductive effort for natural and mitigation plant material. Specifically, we addressed the following questions: 1) What visits *D. multicaulis* diurnally? 2) Might the plant self without a vector? If so, what is the mechanism of

this selfing? 3) How constant are the visitors to *D. multicaulis*? 4) How much nectar is produced per flower in *D. multicaulis*? 5) What is the reproductive output in the natural and mitigation populations of *D. multicaulis*? 6) How viable are the seeds produced by plants in the natural versus the mitigation populations? 7) Do transplanted natural and mitigation population seedlings survive and reproduce during the first year?

## MATERIALS AND METHODS

*Dudleya multicaulis* is a member of the succulent family Crassulaceae (the stonecrops). Detailed descriptions of the family, genus, and this specific species can be found on line ([http://ucjeps.berkeley.edu/cgi-bin/get\\_JM\\_treatment.pl?3284,3295,3324](http://ucjeps.berkeley.edu/cgi-bin/get_JM_treatment.pl?3284,3295,3324)). *Dudleya multicaulis* is an herbaceous perennial that comes up each year from over wintering underground corm-like tuberous caudices. *Dudleya multicaulis* occurs on heavy clay and rocky soils in barren areas among coastal sage scrub and chaparral communities (Munz 1974) and was originally found from coastal Los Angeles County south to Camp Pendleton and inland to Riverside and San Bernardino Counties, in California.

In *D. multicaulis*, the flowering stalk is often multiple-branched and bears lemon yellow flowers. According to Munz (1974), the many-stemmed *Dudleya* flowers in May–June; however, both BLM (2005) and Marchant et al. (1998) give the blooming season as April–July, which is more consistent with our observations. Nascent inflorescences of *D. multicaulis* start to appear in March and April, each beginning as a pink-stemmed stalk produced near the center of the plant. Each primary stalk usually forked at least once, producing two secondary stalks. Some secondary stalks fork again, producing tertiary stalks. A single flower appears at the first fork and is the first to open. From there, blooming continues up the stalk in succession (Fig. 1). Flower “1” opens first, reaches peak bloom, if pollinated tending to develop a reddish tinge on the petals, and begins to form fruit. Relative ages of each inflorescence can be estimated by examining the condition of their flowers. Young inflorescences have their lowest flowers open and none in fruit. Intermediate-aged stalks have open flowers mid-way along the inflorescence branches with the lowest in fruit. Older inflorescences are in flower at the tips (“n” flowers) and in fruit below.

In late summer or fall, follicles dehisce and fall off of the plant. Seeds are about 0.8 mm long. Caesares and Koopowitz (unpublished) report that the average flower, with its five follicles, produces about 12 seeds, of which approximately 52% were viable when germinated under nursery conditions. All aboveground parts senesce in

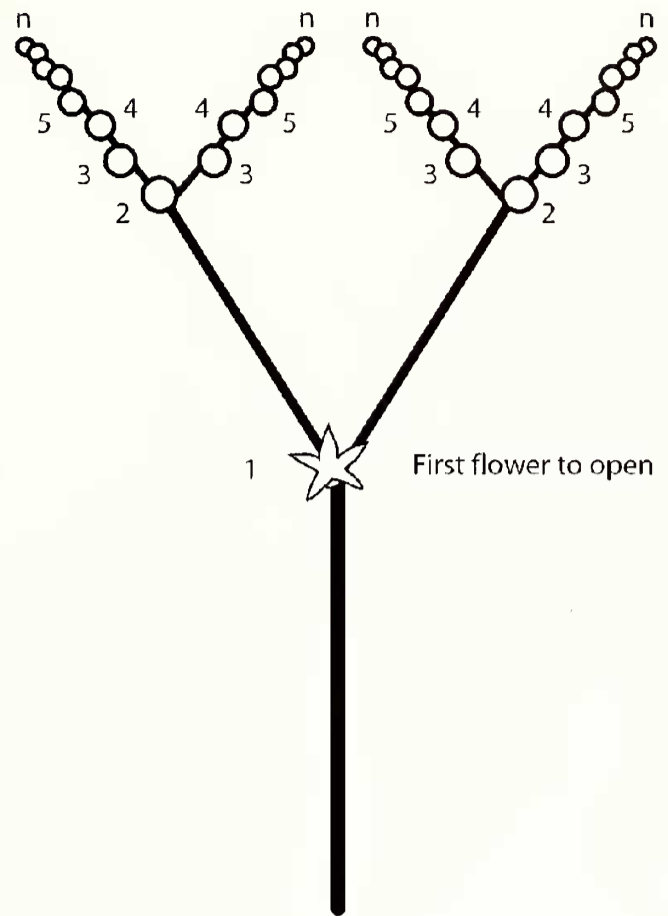


FIG. 1. Blooming sequence. Generalized flowering pattern of *Dudleya multicaulis*. 1 = first flower to open, 2 = second flowers to open, 3 = third flowers to open, 4 = fourth flowers to open, 5 = fifth flowers to open, n = last flowers to open. Flowers 6 through (n-1) were intentionally left unlabeled.

summer, leaving only the dried inflorescence in place. The small stature and growth habit of this species make it difficult to see and, as a result, it is easily overlooked by botanical surveyors.

## Study Sites

*Primary study site.* The site where the pollination studies were conducted from 13 to 15 May 2005 is located in the Santiago Hills just east, south east of Irvine Regional Park, Orange Co., California. Here a rather large population of *D. multicaulis* occurs on a northwestern facing slope near an abandoned stretch of the old Santiago Canyon Road. A series of four *D. multicaulis* subpopulations were initially delimited for study. Beginning lower on the slope and proceeding to the top of the hill, the four subpopulations were identified as follows: subpopulation C — the control site that was used for the collection of *D. multicaulis* floral visitors (insects captured at this site were used for identification and for pollen constancy studies). Subpopulations 1, 2 and 3a were dedicated for use in the dawn-to-dusk pollinator observation studies. On the second day of the dawn-to-dusk studies, subpopulation 3a was replaced by a nearby subpopulation (3b), which contained a larger number of *D. multicaulis* plants in flower. Site 3b was located at the top of the hill adjacent to the fence line separating

TABLE 1. GPS COORDINATES FOR THE SUBPOPULATIONS STUDIED AT SANTIAGO HILLS, ORANGE COUNTY, CALIFORNIA.

Subpopulation	Latitude	Longitude
C	33°47.242'N	117°44.802'W
1	33°47.240'N	117°44.792'W
2	33°47.226'N	117°44.782'W
3a	33°47.217'N	117°44.778'W
3b	33°47.215'N	117°44.772'W

the overall study site from a Toll Road (SR-261). GPS coordinates for these sites are presented in Table 1.

*Ancillary study sites.* The mitigation sites in Weir Canyon (GPS coordinates 33°48.784'N, 117°44.767'W) and Limestone Canyon (GPS coordinates 33°43.522'N, 117°39.721'W) were examined on 15 May 2005 and 27 May 2005, respectively, to determine how many of the mitigation plants were flowering. These plants were counted and later (on 9 July 2005 at Weir Canyon and on 14 July 2005 at Limestone Canyon) examined to determine how many of the flowers on these plants produced one or more follicles and whether these fruits contained one or more fully formed seeds. Fully formed seeds were assumed to be viable and were later utilized in the germination studies.

### Pollination

*Pollinators/visitors—Dawn-to-dusk observations.* To determine pollinator behavior, diversity, and the relative importance of each of the major pollinator groups, a series of dawn-to-dusk surveys was conducted during the peak *D. multicaulis* bloom at the Santiago Hills study site from 13 through 15 May 2005. Peak bloom is herein defined as the time when greater than 50% of the plants were in flower. Pollinators visiting *D. multicaulis* plants were observed during at least 10 min out of each hour beginning on the hour after sun up and continuing throughout the day until 50 min after the hour before sun down. This survey involved three consecutive days of observation.

At the study site, each of the three subpopulations (1, 2, and 3) was selected on the basis of the ease with which field assistants could observe a sizeable number of plants. Two observers were employed to conduct simultaneous observations at each subpopulation during the three days of study. Each person observed and recorded the visitors to *D. multicaulis* plants and the number of flowers each visited in the initial subpopulation (e.g., 1) during the first 10 min of each hour. The observers then had 10 min to move to the second subpopulation (2) where visitors and visits were observed and recorded from 20 min after the

hour until half past the hour. Finally, these same observers rotated to the third subpopulation (3) and repeated the process from 40 min after the hour until 50 min after the hour. Each day the starting subpopulation was rotated so that, during the three-day period, each of the three study plots or subpopulations was the first to be sampled at the start of the observations for that day.

A visitor was defined as any organism that actually landed on and came into contact with the anther(s) and/or the stigma(s) of the flower. Visits were defined as the number of times that a particular visitor landed on one or more flowers of *D. multicaulis* and probed that flower for nectar and/or pollen. Data were subsequently analyzed in terms of number of visitors and visits.

*Pollinator/visitor collection and identification.* Representative samples of visitors were collected from 13 to 15 May between 9:00 and 18:00 at subpopulation C. Organisms seen visiting three or more flowers were captured in an insect net or by using a blowing aspirator and placed in killing jars charged with ethyl acetate. Each specimen was returned to the laboratory, pinned and prepared for identification and pollen sampling. Hymenopteran samples were taken to Roy Snelling at the Natural History Museum of Los Angeles County for identification. All other visitors were identified, at least to order, by the investigators.

*Pollen analysis.* Each captured visitor was examined under a Bausch and Lomb dissecting microscope to determine if pollen was present on the visitor and, if so, where it was located. A 3 cm piece of double-sided Scotch® tape with one end cut to a point and that end was used to pick up any available pollen from the visitors under the dissecting scope. Once the pollen had been transferred from the visitor to the double-sided tape, the tape was placed on a 7.62 cm × 2.54 cm × 1 mm glass microscope slide. One or two drops of cotton blue (1% aniline blue in lactophenol) were added to stain the pollen grains and the slide allowed to sit for at least 24 hrs for the stain to take effect. Slides were then viewed under a Leitz compound microscope and any pollen grains present were identified as either *D. multicaulis* pollen (no other species of *Dudleya* were in flower in the local area) or foreign pollen (using pollen reference slides). The number of plant species and pollen grains found on each individual visitor was used to determine which pollinators carried the pollen of *D. multicaulis* and how constant they were to *D. multicaulis*. A minimum of 100 pollen grains were examined for each specimen, except in the case of two of the flower beetles, where only 10 and 23 total pollen grains were located and identified. Pollinator constancy was defined on a percentage basis. The higher the percentage

of one pollen species in a sample, the more specific that pollinator was to that particular plant species. A pollinator was considered to be "constant" when that pollinator visited a given species at least 95% of the time during a single foraging flight.

*Nectar samples.* Near subpopulation 3b, five plants that were in bud but had no open flowers were entirely covered with light colored knee high nylon stockings on 13 May 2005. These stockings served as pollinator exclusion bags and were secured with a twist-tie to create a seal between the bag and the stem of the *D. multicaulis* plant to ensure that no pollinators visited the flowers. After approximately five days, these five plants were brought back to the laboratory where the presence of nectar was subsequently sampled using 1  $\mu$ l Drummond "microcaps" disposable micro-pipettes (Drummond Scientific Company, Broomall, PA). On 18 May 2005, at least 3 newly-opened flowers on each of the five plants were probed with the micro-pipettes to determine if nectar was being produced and, if so, how much was being secreted per flower.

### Reproduction

*Reproductive output.* Between 9 July and 14 July 2005, plants at the Santiago Hills study site, as well as the Weir Canyon and Limestone Canyon mitigation sites, were examined to determine the number of flowers produced per inflorescence and how many of those flowers contained one or more follicles. This was done to determine if there were differences in flower production per inflorescence among the sampled sites and to determine the percentage of fruit set per flowers produced. Also, while examining the fruit, mature flowers with fruit were harvested from the control (C) subpopulation at the Santiago Hills study site, from the natural population at the Weir Canyon site, and from the mitigation plants at Weir and Limestone Canyons. Two sub-samples were examined at the Weir Canyon natural population. The first sample (identified as population 1) was taken from the lower portion of the natural population on the north west facing slope and the second sample (identified as population 2) was removed from plants that co-occurred with the mitigation plants at the top of the same natural population. A total of 10 or 11 flowers were harvested at each site, one each from 10 or 11 different plants, except for the Weir and Limestone Canyon mitigation sites where more than one flower was harvested per plant to achieve a sample of 10 flowers. The number of fully formed seeds per fruit and per flower was determined.

Twenty-five inflorescences, one each from separate plants, were sampled from each of the

subpopulations (C, 1, 2, 3a, and 3b) studied at the Santiago Hills site. At the Weir Canyon site, one inflorescence each from 50 naturally occurring separate plants found on the north west facing slope were examined and one inflorescence each from four of the eight plants that had been spotted and marked with flags on 15 May 2005 were examined; the other four marked plants could not be located. One inflorescence each from seven of the eight plants that had been identified and marked with flags on 21 May 2005 at the Limestone Canyon Site were also examined. The eighth flagged plant at this location could not be located. The seeds harvested from these samples were then submitted to germination tests.

*Seed germination tests.* A total of 208 seeds from the Santiago Hills site, 101 seeds from the Limestone Canyon site, 137 seeds from the Weir Canyon natural occurring plants, and 12 seeds from the Weir Canyon mitigation site, were harvested from the flowers produced by the plants in each of these four sites. Of these, a subsample of 100 seeds (except for the Weir Canyon mitigation site where all seeds recovered were utilized) were placed on moistened 38 lb. 8.9 cm circles of regular seed germination paper (Anchor Paper Company, St. Paul, MN) in 100  $\times$  15 mm Fisherbrand disposable sterile petri dishes (Fisher Scientific, Los Angeles Office, Tustin, CA). A total of 18 petri dishes were utilized as follows: five petri dishes with 20 seeds per dish or 100 seeds per each were prepared for the Santiago Hills, Limestone Canyon, and the Weir Canyon natural sites. Since there were so few inflorescences produced by the mitigation planting at the Weir Canyon site, there were fewer seeds available so only 3 petri dishes with 4 seeds per each or a total of 12 seeds were prepared for the germination tests. The petri dishes were watered with 5 ml of deionized water and placed in individual Ziploc<sup>®</sup> one quart storage bags (A product of S. C. Johnson & Sons, Inc., Racine, WI), labeled with an identification code, and then randomly placed in one of two Percival Model E-30B growth chambers (Percival Scientific, Inc., Perry, IA). Each growth chamber was then set on 11 hr of daylight with 15°C daytime temperature and 10°C nighttime temperature. Germination was monitored daily from 3 October 2005 through 28 November 2005.

*Transplanted seedling survival tests.* A sample of the germinated seedlings from each site was transplanted into 5.5  $\times$  5.5  $\times$  8.5 cm (W  $\times$  D  $\times$  H) black plastic pots filled with potting soil on 5 January 2006 and followed through the growing season of 2006. The potting soil was a mix of an organic fraction (50%) that included peat moss (6 parts by volume) and forest humus (9 parts by volume) and of an inorganic fraction (50%) that included washed plaster sand (6 parts by volume)

## Total Visits - Dawn to Dusk

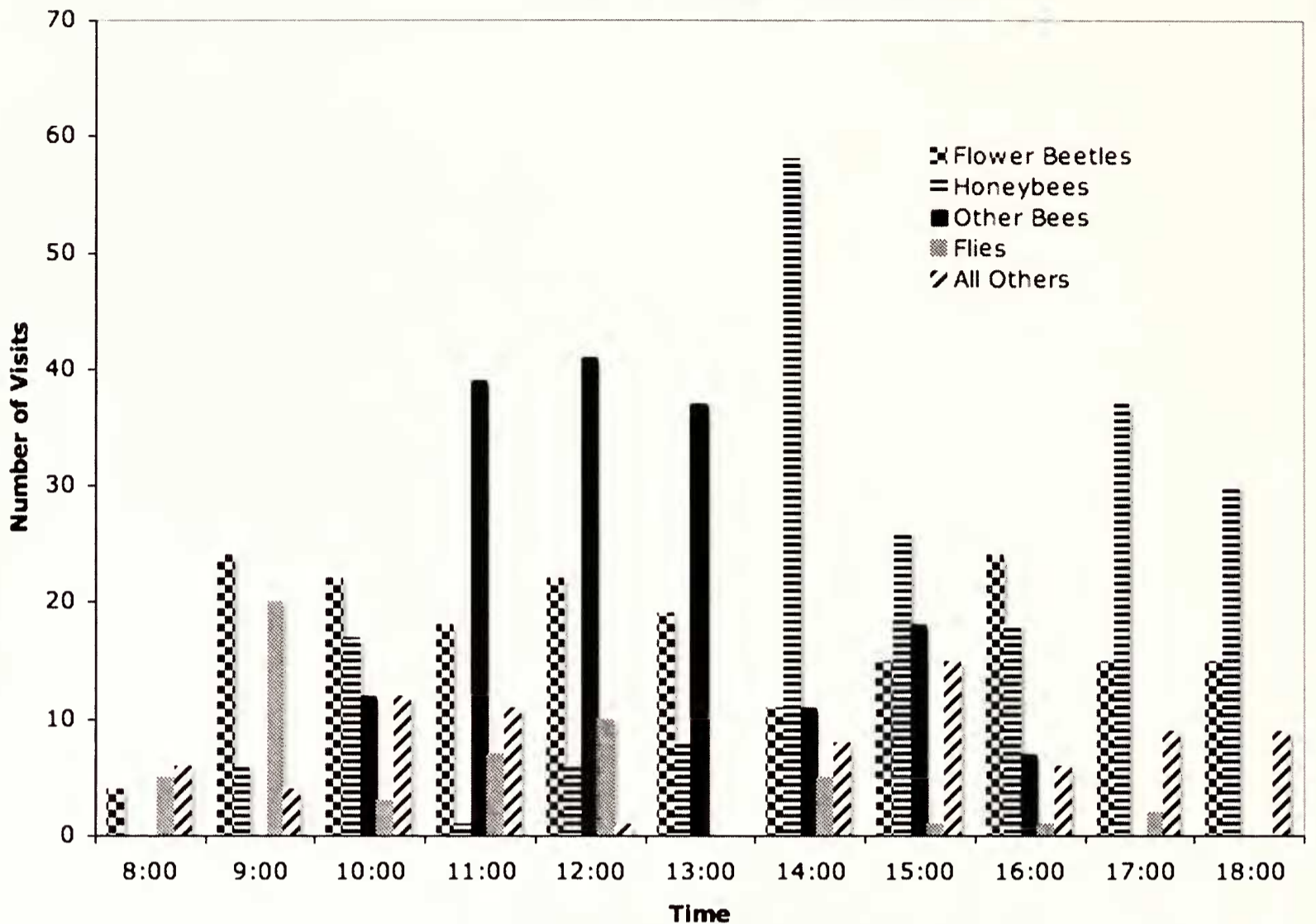


FIG. 2. Total visits by all visitors by time of day for all study plots (1, 2, 3a, and 3b) for 13–15 May 2005 combined.

and pumice (9 parts by volume). Time-released fertilizer was added at the rate of 4oz/10 gallons soil mix and dolomite (Ca and  $MgCO_3$ ) at 5oz/10 gallons of soil mix. The time released fertilizer used was Sierrablen 18N:7P:10K + Fe.

The potted plants were placed outdoors and watered daily or as needed following rains. They were monitored for survival and reproduction at the end of the growing season on 23 June 2006.

#### Statistical Analyses

Sites were compared using one-way analysis of variance (ANOVA) or a Kruskal-Wallis Rank Sum Test when required for flowers produced per plant, fruit-set per flower, seeds per flower, seed germination, and survival of transplanted seedlings derived from the germination tests. Tests were done using Excel.

## RESULTS

### Pollination

*Pollinators/visitors.* Hymenopterans included the European honey bees (*Apidae*, *Apis mellifera* L.), two bee species in the family Halictidae

(*Halictus tripartitus* Ckll. and *Lasioglossum* [*Dialictus*] sp.), one bee species in the family Megachilidae (*Hoplitis grinnelli* [Ckll]), and possibly two separate species of ants (all Order Hymenoptera). Other visitors included soft-winged flower beetles (Melyridae, Dasytinae, possibly *Lystrus* sp.) and weevils (both Order Coleoptera), several flies including those in the family Syrphidae as well as other families (Order Diptera), with only a few individuals each of true bugs (Order Hemiptera), leafhoppers (Order Homoptera), and flower mites (Order Acari). Of these visitors, the most frequent and/or most important (judging by behavior within the flowers that indicated a high probability of successful pollination) included the flower beetles, honey bees, and bees in the families Halictidae and Megachilidae.

*Dawn-to-dusk observations.* The results of the total dawn-to-dusk observations are summarized on a diurnal basis (Fig. 2). It is interesting to note that flower beetles were found visiting the flowers during the entire daily observational period. Non-native European honey bees tended to be more common in the afternoon hours, whereas the native solitary bees tended to frequent the flowers earlier in the day. Flies seemed to visit the

flowers early in the morning. All other visitors appeared to show a bimodal distribution arriving in the morning and then again in the afternoon.

The frequency of visits by the various groups of potential pollinators (visitors) for all study plots and all three days of observation was combined. There was considerable variation among the three subpopulations regarding the frequency of visits by the various groups, but the total frequency distribution provides a good representation of the overall visits to *Dudleya multicaulis*.

Of the total visits to *D. multicaulis*, flower beetles accounted for 31% of the visits and they represented 56% of the visitors. European honey bees and other bees, in contrast, made 27% and 19% of the visits respectively, but were represented by only 8% and 8% of all visitors, indicating that the bees were typically visiting more than one flower per foraging bout on *D. multicaulis*. Flies and all others accounted for 9% and 14% of all visits to *D. multicaulis*, but had 10% and 18% of all visitors respectively. As in the case of the visits at each of the subpopulation study sites, there was considerable variation in the relative frequency of the various groups of visitors at each study site.

Considering each subpopulation individually, 78% of the visits observed in subpopulation 1 were from flower beetles, whereas none of the other groups contributed more than 8% of the visits. Further, 84% of all *D. multicaulis* floral visitors were flower beetles.

Visits to flowers of *D. multicaulis* at subpopulation 2 were more equally distributed among the various pollinating groups with 32% of all visits being by other bees, 28% by flower beetles, and 36% by all others. Flies and European honey bees accounted for only 3% and 1% of the visits respectively. In terms of visitors, flower beetles represented 32% of the visitors, whereas other bees and all others accounted for 31% and 31% of the visitors respectively. Flies and European honey bees accounted for 3% of the visitors each.

Since we used two separate plots for subpopulation 3, two distinct patterns were observed for the visits and visitors to subpopulations 3a and 3b. Subpopulation 3a, which was utilized only on 13 May 2005, showed 44% of all visits were by the all others group, 31% by flower beetles, 17% by flies, 6% by other bees and 2% by European honey bees. An examination of the visitors for the same subpopulation shows that 40% of the visitors were in the all others group, 36% were flower beetles, 15% were flies, 6% were other bees, and 3% were European honey bees.

In contrast, visits to *D. multicaulis* flowers in subpopulation 3b, which was observed 14–15 May 2005 showed that 39% of the visits were by European honey bees, 32% by other bees, 12% by

flower beetles, 11% by flies, and only 6% by all others. This represents quite a contrast with the visits observed at subpopulation 3a and may simply reflect the consequence of a much larger floral display present at subpopulation 3b in comparison to 3a. Data for visitors of the various groups at this subpopulation (3b) show that members of some of the groups made multiple visits per foraging bout (e.g., honey bees and other bees with only 14% and 25% of the visitors), whereas individuals of other groups of visitors usually visited only a single flower per foraging bout.

*Pollen analysis.* Pollen taken from the sampled visitors was identified. The three bee species (European honey bee,  $n = 6$  and halictid bee species,  $n = 5$ , exhibited an average floral constancy of 98.7 and 99.7% with standard deviations of 2.61 and 0.67 respectively. The same was also true for the soft-winged flower beetle (Melyridae,  $n = 4$ ), which had an average constancy of 74.8, but the standard deviation was much higher at 26.6.

*Nectar analysis.* *Dudleya multicaulis* plants produced an average of 0.12  $\mu\text{l}$  per flower. Average nectar production per the five sampled plants varied from 0.08  $\mu\text{l}$  to 0.17  $\mu\text{l}$  per flower. Nectar production per flower was minimal.

## Reproduction

*Flower and fruit production.* Data regarding the number of flowers produced per inflorescence and the percentage fruit-set for sampled plants at the various study sites/subpopulations are presented in Table 2. Although there were no significant differences among sites ( $F_{8,9} = 0.94$ ,  $P > 0.52$ ), the subpopulations at Santiago Hills generally produced a few more flowers per inflorescence than either of the mitigation populations at Weir Canyon or Limestone Canyon. Of the latter two, the Weir Canyon mitigation site, which was located within approximately 30 meters of a natural population of *D. multicaulis*, produced a few more flowers per inflorescence than those at Limestone Canyon, a population which was separated from a natural population of *D. multicaulis* by well over 2 km (Table 2).

There were also no significant differences for average fruit-set among sites (Kruskal-Wallis Rank Sum Test value = 4.34,  $P > 0.82$ ). However, the average fruit-set was always greater than 85%. The range in fruit-set varied among and within the *D. multicaulis* populations from a low of 60% in the Limestone Canyon mitigation population to 100%, a high value that was found in all studied populations including the Limestone Canyon mitigation population.

TABLE 2. NUMBER OF FLOWERS PRODUCED PER INFLORESCENCE AND PERCENTAGE FRUIT-SET FOR PLANTS IN THE VARIOUS STUDY POPULATIONS AND SUBPOPULATIONS. n = the number of inflorescences sampled. Ave. fl. = average flower number, SD fl = standard deviation for that average, and R fl = range of number of flowers produced per inflorescence. Ave. % fr. = average percentage fruit set, SD fr = standard deviation for that average, and R fr = range of fruit set per flowers produced on inflorescences.

Study site/subpopulation	n	Ave. fl.	SD fl	R fl	Ave. % fr	SD fr	% R fr
Santiago Hills – subpop. C	25	12.2	5.43	6–31	91.6	7.14	78.6–100
Santiago Hills subpop. 1	25	14.6	6.33	5–33	94.4	6.20	80.0–100
Santiago Hills subpop. 2	25	13.2	5.01	6–27	86.9	9.16	64.3–100
Santiago Hills subpop. 3a	25	20.0	7.54	10–37	92.2	5.89	81.1–100
Santiago Hills subpop. 3b	25	34.5	21.42	11–94	92.5	5.99	80.0–100
Weir Canyon natural pop. 1	50	11.5	7.21	2–35	89.7	11.10	60.0–100
Weir Canyon natural pop. 2	21	11.2	7.27	2–29	94.2	7.69	71.4–100
Weir Canyon mitigation plants	4	5.3	1.71	3–7	92.3	9.0	83.3–100
Limestone Canyon mitigation plants	7	5.6	1.90	2–8	87.0	14.7	60.0–100

*Seed production.* No significant differences in average seed production per site was found among populations ( $F_{1,6} = 3.65$ ,  $P > 0.10$ ). The average seed production per flower varied by population from a low of only 0.3 in the Weir Canyon mitigation population to a high of 5.4 in the Santiago Hills subpopulation C.

The Santiago Hills population produced the highest number of fully-formed seeds per flower, (each flower having 5 separate fruits [follicles]), followed by the natural population at Weir Canyon. The mitigation plants at the Limestone Canyon site produced the next highest number of seeds per flower, whereas the Weir Canyon site produced the fewest number of seeds per flower. In fact, only one plant of the four plants sampled from this latter site contained any seeds.

*Seed germination.* The percentage of seeds germinating by site was not significantly different from one another (Kruskal-Wallis Rank Sum Test value = 0.17,  $P > 0.98$ ). An examination by site showed that at least 25% of the seeds had germinated after the first 48 hr of the tests. Percent germination at each site was quite good with all sites ranging from 62% at the Limestone Canyon Mitigation Site, to 65% at the Santiago Hill Subpopulation C, to 83.3% at the Weir Canyon Mitigation Site, to a high of 85% at the Weir Canyon Natural Population. It is interesting to note that the two Weir Canyon sites had the highest germination percentages. This may be important to the ultimate survival of the population at the Weir Canyon mitigation site since so few seeds were produced by the meager number of surviving mitigation plants at that site.

*Transplanted seedling survival and reproduction.* Transplanted seedling survival to successful reproduction did not differ significantly by site ( $F_{3,4} = 0.29$ ,  $P > 0.83$ ). However, of all transplanted seedlings from all the study sites, a minimum of 25% of them survived to flowering and fruit production. The lowest survival was found in the Limestone mitigation site (at 25%)

and the highest was in the Weir Canyon mitigation plants (37.5%). Conversely, between 62.5% and 75% of the transplanted seedlings died prior to maturity, indicating a relatively minimal transplantation survival rate even under the nearly ideal conditions used during this study.

## DISCUSSION

Pollination by biotic agents is a mutualism that has the potential to control important aspects of plant reproduction and can play a critical role in the survival and management of rare species (Schemske et al. 1994; Kearns and Inouye 1997; Bernardello et al. 1999; Kaye 1999; Timmerman-Erskine and Boyd 1999; Spira 2001). Therefore, a knowledge of the pollination biology of any rare species takes on greater importance given the potential effect of such interactions can have on the continued existence of the rare species.

### Pollinator Activity and Floral Constancy

Observations of pollinator activity were only made during the peak time of flowering. Future studies should examine pollinator activity during early, mid- and late flowering periods to determine the total spectrum of visitors (potential pollinators) and how it may or may not vary from the beginning to the end of the blooming period. The observations of pollinators within the current time frame revealed that the primary pollinators as judged by their behavior at the flowers (which included contacting the anthers and/or stigmas during a floral visit) were European honey bees and bees in the families Halictidae and Megachilidae, although flower beetles were usually the most abundant visitor at most of the plots. Six specimens of flower beetles were examined to determine if they carried *Dudleya multicaulis* pollen and this pollen of *D. multicaulis* was found on four of those individuals. Given the observed behavior of flower beetles within the flowers, the most likely role

they play in the pollination process of *D. multicaulis* is in selfing within a flower.

Our data support the suggestion that *D. multicaulis* has adopted a generalist pollination strategy (see Waser et al. 1996; Gomez and Zamora 1999, for a more detailed overview of this strategy). Plants living in fluctuating environments such as the southern California Mediterranean climate have to deal often with substantial annual variation in rainfall. Such variability in rainfall in seasonally dry environments can have a substantial effect on the number of plants that emerge from dormancy, grow to maturity, successfully flower and set fruit (Beatty 1974). The generalist pollination strategy then provides a mechanism to ensure some successful reproduction even in years in which plant population levels, flowering resources, and possibly pollinator numbers and diversity are reduced by lack of rainfall (Waser et al. 1996; Aigner 2001, 2003, 2005; Gomez and Zamora 2006). One of the potential consequences of a reduction in the diversity of potential pollinators in dry years is the potential loss of pollinator species that are more likely to effect outcrossing between plants. This occurs because flowers of species whose population levels fall low enough to reduce the floral rewards to levels that do not meet the energetic needs of the pollinators that are likely to facilitate outcrossing, such as many species of bees (Sih and Baltus 1987; Jennersen and Nilsson 1993; Conner and Neumeier 1995). As a result, selfing is more likely since remaining pollinators are ones (like flower beetles) that require fewer resources to meet their energetic needs.

#### Fruit Set

The total number of visitors seen visiting the flowers of *D. multicaulis* during our study was relatively small. Although fruit set varied among the subpopulations investigated, the differences were not significant. When we harvested inflorescences to determine fruit set, we found that nearly every flower had five fully developed follicles, indicating that reproduction did not seem to be pollinator limited. Fruit set was so high (in every case over 85%) that we suspected *D. multicaulis* might be at least partially self compatible (Sutherland 1986). Sutherland (1986) reviewed the fruit/flower ratios of many plant species and determined that high ratios, certainly those above 33%, were found in plants that were at least partially self-compatible. In view of the small number of visitors observed during this study and the high fruit set, we suspected that *D. multicaulis* may not require a pollinator to effect fruit production (hence self fertile, see Harding et al. 1974; Lloyd and Schoen 1992).

#### Nectar Production

We found that nectar production per flower was low in comparison to species of *D.* reported for the subgenus *Dudleya* (Levin and Mulroy 1985). This reduced nectar production is a characteristic of species that do not to rely on pollinators to effect successful reproduction (Levin and Mulroy 1985).

#### Self Fertility

We closely examined the flowers of *D. multicaulis* and found some interesting features that may contribute to the high fruit-set in this species. Selfing without a vector within a single flower may occur. Each flower has 10 stamens, five alternate and five opposite the petals. The five pistils begin to fold back into the groove of the V-shaped petals and their styles begin to elongate. During this process, the stigma becomes receptive to pollen deposition. If the receptive stigma does not receive pollen via normal pollinator facilitated transfer, the virgin stigma can pick up pollen as the style elongates and pushes the stigma past the anther on the stamen opposite the petal. If pollen remains on these anthers opposite the petals, selfing without a vector can occur if the pollen remains viable.

The observed floral morphology suggests that *D. multicaulis* may not require a pollinator to effect fruit production and may be able to get pollen into contact with receptive stigmas without the involvement of biotic agents. We emphasize that this is a tentative conclusion and requires further data from additional experimental procedures before it can be confirmed. Specifically, bagging or exclusion experiments are required to determine the breeding system of *D. multicaulis*. If seed is produced by selfing without a vector, then germination and seedling fitness tests should be completed. Further, any seeds produced in the bagging experiments that result from selfing with a vector (transfer of pollen from a flower on a plant to another flower on the same plant) or outcrossing should also be tested for germination and seedling survival. Levin and Mulroy (1985) found that significantly more seed was produced by outcrossing in species in the genus *Dudleya* subgenus *Dudleya* than by selfing with or without a vector and that seedlings from outcrossed seeds also survived better than those produced by either mode of selfing.

#### Reproductive Output

Reproductive output, as judged by seed production was not significantly different among sites and was reasonable at all sites except the mitigation plants at Weir Canyon. Of the four plants sampled from that group, only one



produced any fully formed seeds. This finding suggests that selfing does not always occur. This group of plants bears watching and may not survive with such low reproduction. Average seed production per flower also did not vary significantly among our study populations (ranging from a low of 0.3 to a high of 5.4 seeds per flower) and were much lower than the approximately 12 seeds produced per flower found by Casares and Koopowitz (unpublished).

#### Seed Germination and Seedling Transplantation

Tests were completed on the seeds produced by *D. multicaulis* to see if they will germinate and result in successful offspring. The seed extracted from plants from each of the four study sites germinated quite well and the percent germination was not significantly different among sites. Germination ranged from 62% for the Limestone Canyon mitigation site to 85% for the Weir Canyon natural site. Our germination results are higher than those found by Casares and Koopowitz (unpublished) who recorded a germination rate of about 52% under nursery conditions. It would appear that seeds produced by all plants demonstrate sufficient viability to ensure successful seed reproduction. Further, when the germinated seedlings from these seeds were transplanted into pots and placed out-of-doors under relatively normal conditions, except for regular watering, between 25% (Limestone Canyon mitigation site) and 37.5% of the plants (Weir Canyon mitigation site) survived and successfully produced one or more seeds by the end of the first year. It should be noted, however, that between 62.5% and 75% of all transplanted seedlings died during this first year when they were grown under nearly ideal conditions. Transplanting of seedlings or adult plants to new locations would not seem to be a viable alternative to sowing harvested seed as a mitigation measure for this species. It should be noted that the two mitigation sites were dissimilar in that the Weir Canyon mitigation site was within approximately 30 m of an existing natural population, whereas the Limestone Canyon mitigation site was quite remote from any existing natural population of *D. multicaulis* (ca. 2 km).

#### Reproductive Strategies

Wilken (unpublished) investigated the reproductive strategies of *D. nesiotica* (Moran) Moran, another member of the subgenus *Hasseanthus* and concluded that it is self-compatible but requires a vector to facilitate reproduction. Levin and Mulroy (1985) studied the pollination biology of several species in the genus *Dudleya* subgenus *Dudleya* and found that two of the

three major groups of species in this subgenus demonstrated a significant degree of self-fertility. They attributed this to unreliable pollinators and/or environmental unpredictability. By unreliable pollinators, they meant pollinators that varied considerably in abundance both temporarily and spatially (Levin and Mulroy (1985). In our study, pollinator abundance appeared to be minimal. It could be that the past few drought years have had a negative effect on insect populations. It may take a few wet years for insect populations to return to normal.

Environmental variability, and thus unpredictability of resources and pollinators, has certainly been a factor in the development of southern California ecosystems as rainfall varies considerably in both amount and pattern from year to year. Therefore, if self-fertility is found to be a significant mode of reproduction in *D. multicaulis*, then it may represent an adaptation that increases overall reproductive success in habitats like the coastal sage scrub community and for species like *D. multicaulis* (Moeller 2006). However, it again needs to be emphasized that in *Dudleya* subgenus *Dudleya*, selfing with a vector and outcrossing both resulted in more seed production and, in the case of outcrossed seed, better fitness of the seedlings (Levin and Mulroy 1985). Similar seed set results were also found for *D. nesiotica* in that it produced about the same fruit set when manually selfed (22.1 seeds per flower) or when outcrossed (20.3 seeds per flower). However, if emasculated and unpollinated, no fruit set occurred (Wilken unpublished). Wilken (unpublished) provides no data relative to the possibility that self-fertility can occur within flowers in time, if vector facilitated pollination does not occur before the senescence of the flower.

Self pollination is also prevalent in habitats with short growing seasons (Runions and Geber 2000; Mazer et al. 2004). *D. multicaulis* occupies such a habitat, one characterized by extreme annual variation in rainfall, which tends to favor small flowers (Strauss and Whittall 2006). Smaller flowers like those found in *D. multicaulis* increase the likelihood of selfing because of the close proximity of the anthers and stigmas (Snell and Aarssen 2005). This association of small flower size and variable water availability has been shown to increase selfing in several annual plants genera (Guerrant 1989).

The breeding biology of a rare species is a very important issue that requires careful consideration by decision makers when movement of plants is required for mitigation purposes. For example, if a rare plant requires no pollinator and still sets abundant seed, and assuming such seed germinates and the progeny survive, then, at least in the short term the sowing of this seed may increase the probability of successful mitigation

in cases where plants must be removed from a site. This appears to be the best option for *D. multicaulis*.

However, selfing can have more long-term consequences that include increased inbreeding depression and increased homozygosity in the interbreeding population and, thus, decreased genetic variation at the colony scale. One of the goals of many conservation programs is to maintain genetic diversity in species that are rare, threatened, or have small population size like *Dudleya multicaulis* (Frankel and Soulé 1981; Simberloff 1988). For this reason, genetic studies of rare plants should be completed whenever possible. Information from these studies can establish much about the species that will assist in its successful management (Ellstrand and Elam 1993).

### Genetic Structure and Selfing

In a previous study of the genetic structure of *D. multicaulis* by Marchant et al. (1998), they concluded that there is little evidence for significant gene flow between populations and that local populations tended to show heterozygote deficit. They also indicated that reduced genetic variability within populations of *D. multicaulis* might be a consequence of founder effects and subsequent mating among relatives. We would add that selfing should also be considered. In this regard, Marchant et al. (1998) did note that *D. multicaulis* can self, but indicated that they had not investigated if selfing in *D. multicaulis* lowered the fitness of the progeny. Data from Levin and Mulroy's study (1985) of *Dudleya* subgenus *Dudleya* suggest that lowered fitness may indeed be the case.

Marchant et al. (1998) additionally state that variation among *D. multicaulis* populations tended to be significant, further indicating that gene flow by either pollen transport or seed dispersal was limited. How far apart, then, must *D. multicaulis* populations be for genetic isolation to be significant? That remains to be determined for *D. multicaulis*, but an interesting recent study by Boose et al. (2005), examined genetic variation in *Navarretia leucocephala*, and concluded that distances of 1100 to 1800 m were often sufficient to result in significant genetic differentiation between populations. Therefore, for species like *D. multicaulis* with limited pollen and seed dispersal capabilities, it is quite probable that significant interpopulational variation in genetic structure could occur at these distances or less.

However, it may be that selfing is a key component in the survival of this species. A recent paper by Morgan et al. (2005) demonstrated, using models, that plants with population densities that vary annually with environmental conditions (like *D. multicaulis*) may avoid extinc-

tion by increased reliance on autogamy, especially when they are pollinated by generalist pollinators (as is the case with *D. multicaulis*). Further, their models also showed that delayed selfing is always favored. At least selfing without a vector appears to occur in *D. multicaulis* only if pollinator services are not forthcoming since *D. multicaulis* is protandrous. If our model of selfing without a vector is shown to be a functional mode of reproduction in *D. multicaulis*, it may mean that newly established mitigation populations may be able to persist without going extinct because of their ability to self without a pollination vector. In fact, they may be able to persist long enough to develop sufficiently large plant populations to attract the generalist pollinators required to facilitate outcrossing and increase genetic diversity (Jarne and Charlesworth 1993).

### CONCLUSIONS

There is much more research to be done to elucidate the reproductive biology of *Dudleya multicaulis* to provide the background data required to increase the probability of the successful preservation of this species. However, we suggest that transplantation of plants to new sites may not be as good a mitigation measure as seeding the new sites with seeds derived from those plants. It should be noted that our germination tests were completed under controlled conditions suggesting that artificial watering following seed inoculation of a new location may be necessary to ensure adequate germination and survival.

### ACKNOWLEDGMENTS

We thank Angela Knips for her assistance throughout the project, Frank Wegscheider for his field assistance, Senta Breden and Katie Levensailor for all the hours they spent observing visitors to Dm, and Kelly Argue for all the hours related to the laboratory studies of germination and the greenhouse study of seedling survival and reproduction following transplanting. Unless otherwise noted, all photographs and line drawings in this report were done by Robert L. Allen. Special thanks to Roy Snelling of the Natural History Museum of Los Angeles for the hymenopteran identifications. We gratefully acknowledge the contract grant support provided to C. E. J. by the Irvine Company through a subcontract from LSA Associates, Inc., Irvine, California.

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